

Application No.: 10/081,118

Docket No.: 20198-00059-US

AMENDMENTS TO THE CLAIMS

1. (currently amended) ~~Reagent A reagent for the identification~~ identifying and counting ~~[[of]] biological cells in a sample, in particular in a blood sample, characterized in that it comprises comprising:~~
 - a cell lysing agent ~~selected from comprising~~ comprising at least one detergent in a concentration ~~sufficient to lyse~~ allowing the lysis specifically of a given type of cell in the sample, and
 - a stain ~~designed to mark~~ capable of marking the intracellular nucleic acids of the ~~unlysed remaining cells, for identifying and counting the remaining cells.~~
2. (currently amended) ~~Reagent The reagent according to claim 1, characterized in that~~ wherein the sample is of blood having erythrocytes and the cell lysing agent ~~comprising~~ comprises at least one ionic and/or non-ionic detergent in a concentration capable of lysing erythrocytes.
3. (currently amended) ~~Reagent The reagent according to claim 1, characterized in that~~ wherein the detergent is selected from the group consisting of:
 - primary amines, amine acetates and hydrochlorides, quaternary ammonium salts, and trimethylethyl ammonium bromide;
 - amides of substituted diamines, diethanolamino-propylamine or diethylaminopropylamide, amides of cyclised diethylenetriamine;
 - alkylaryl sulfonates, petroleum sulfonates, sulfonated glycerides;
 - cholamides, sulfobetaines;
 - alkyl glycosides, saponins;
 - polyoxyethylene ethers and sorbitans, and polyglycol ethers.

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4. (currently amended) ~~Reagent~~ The reagent according to claim 1, ~~characterized in that~~ wherein the stain is a fluorescent type stain.
5. (currently amended) ~~Reagent~~ The reagent according to claim 1, ~~characterized in that~~ wherein the stain is capable of combining specifically with the intracellular ribonucleic acid of said unlysed remaining cells whereby and enhancing its the fluorescence once it has combined with the latter of said stain is enhanced.
6. (currently amended) ~~Reagent~~ The reagent according to claim 1, ~~characterized in that~~ wherein the stain is selected from the group selected from:
- thiazole orange or 1-methyl-4-[(3-methyl-2-(3H)-benzothiazolylidene)methyl]quinolinium p- tosylate,
 - thiazole blue,
 - 4-[(3-methyl-2-(3H)-benzothiazolyl-idene)methyl]-1-[3-(trimethylammonium)propyl]quinolinium diiodide,
 - 3,3'-dimethyloxacarbocyanine iodide or 3-methyl-2-[3-(3-methyl-2(3H)-benzothiazolylidene-1-propenyl]benzoxazolium iodide,
 - thioflavine T,
 - ~~thioflavine T,~~
 - the stains SYTO® and TOTO® (TM Molecular Probes),
 - ethidium bromide,
 - propidium iodide,
 - acridine orange,
 - coriphosphine O,
 - auramine O,

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- the stains HOECHST 33258 and HOECHST 33342,
 - 4',6-diamino-2-phenylindole dihydrochloride (DAPI),
 - 4',6-diimidazolin-2-yl)-2-phenylindole dihydrochloride (DIP),
 - 7-aminoactinomycin D,
 - actinomycin D, and
 - LDS 751.
7. (currently amended) ~~Reagent~~ The reagent according to claim 1, ~~characterized in that it~~ which also comprises at least one membrane penetration agent capable of increasing permeability of a cell membrane to promote ~~promoting~~ the penetration of the stain into the unlysed ~~cells to be marked~~.
8. (currently amended) ~~Reagent~~ The reagent according to claim 7, ~~characterized in that~~ wherein the agent promoting membrane penetration is an ionophore compound of the protonophore type, and/or an ionophore of the antibiotic type, or a mixture of ionophores.
9. (currently amended) ~~Reagent~~ The reagent according to claim 1, ~~characterized in that it~~ also which comprises at least one membrane fixing agent present in a concentration of 0.1% to 10% (w/v).
10. (currently amended) ~~Reagent~~ The reagent according to claim 9, ~~characterized in that~~ wherein the membrane fixing agent comprises at least one alcohol ~~and/or at least one~~ [[an]] aldehyde selected from the group consisting of paraformaldehyde and glutaraldehyde, or a mixture of at least one said alcohol and at least one said aldehyde.
11. (currently amended) ~~Reagent~~ The reagent according to claim 1, ~~characterized in that it~~ which also comprises at least one compound selected from the group consisting of a complexing agent, an inorganic salt and a buffer system.

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12. (Withdrawn) Process for the identification and counting of biological cells in a sample, in particular in a blood sample, characterized in that it comprises the following operations:
- mixing and incubating the sample with a reagent according to claim 1 in order to effect, in a single stage, the lysis of cells of a given type, in particular erythrocytes, the staining of the intracellular nucleic acids, and the fixing of the nucleate cells;
 - measuring the resultant solution by flow cytometry using at least two measuring parameters selected from resistive volume, axial luminous diffraction, axial luminous transmission, orthogonal luminous transfusion, and fluorescence; and
 - classifying and counting the nucleate cells in populations by means of the measured parameters.
13. (Withdrawn) Process according to claim 12, characterized in that the resistivity measurement is carried out by means of at least one current selected from a continuous current and a pulsed or alternating current.
14. (Withdrawn) Process according to claim 12, characterized in that the axial luminous diffraction parameter is at least one parameter selected from small angle diffraction and large angle diffraction.
15. (Withdrawn) Process according to claim 12, characterized in that the classified nucleate cells are either mature or immature, normal or abnormal cells.
16. (Withdrawn) Process according to claim 12, characterized in that the classification of the nucleate cells is carried out by means of a multidimensional analysis software program, with or without the use of a software or other neuronal technique.

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17. (Withdrawn) Process according to claim 12, characterized in that the sample is a sample of human or animal blood.
18. (Withdrawn) Process according to claim 12, characterized in that the sample is a sample of biological fluid or a suspension of cells of human or animal origin.

Please add the following new claim:

19. (New) A reagent for identifying and counting blood cells having nucleic acid in a sample having erythrocytes, comprising: a cell lysing agent consisting essentially of a solution having at least one non-ionic detergent in a concentration sufficient to lyse specifically erythrocytes, an ionophore, and a stain capable of marking the nucleic acids of unlysed remaining cells.